PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Rubin et al.

Application No.: 10/719,554

Filed: November 21, 2003

For: ORAL COMPOSITION CONTAINING NSAIDS AND ESSENTIAL OILS

Confirmation No.: 3555

Examiner: B.Y. Kwon

Group Art Unit: 1614

Docket: 4727-C2-03-DCL (1321-10)

Dated: January 18, 2007

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail, postpaid in an envelope, addressed to: Commissioner for Patents, Alexandria, VA 22313

Dated: January 18, 2007

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Declaration of Pauline C. Pan Pursuant to 37 C.F.R. §1.132

I, Pauline C. Pan, do hereby declare as follows:

1. As of the date of this Declaration, I am currently employed as a Research Fellow in the Oral Care-Technology Platform/Development division of Johnson & Johnson (previously Pfizer Consumer Healthcare, and previously Warner-Lambert Company) in Morris Plains, NJ. Prior to employment as a research biologist at Johnson & Johnson/Pfizer Consumer Healthcare/Warner-Lambert Company, I was employed with Pennwalt Corporation in mgr immunopharmacology and I was a visiting research fellow in molecular biology/biochemistry at UMDNJ. I received a Bachelor of Arts in biology from Bryn Mawr College, a Masters in biological sciences from Stanford University, a PhD in microbiology-biochemistry from Rutgers University. I also

completed a post-doctoral fellowship in cell biology at Princeton University and I was a member of the research faculty in cell biology at Princeton University.

2. I am a joint inventor of the subject matter of the above-identified patent application.

3. I have reviewed the Examiner's Office Action dated August 18, 2006, rejecting the claims of the subject application under 35 U.S.C. §103(a) over U.S. Patent No. 5,723,106 to Buch et al. (hereinafter "Buch") in view of U.S. Patent No. 5,294,433 to Singer et al. (hereinafter "Singer") and U.S. Patent No. 6,194,462 to Giorgetti (hereinafter "Giorgetti"). I also have reviewed these cited patents.

4. Buch is related to reduced alcohol antiseptic mouthwash compositions. The compositions include a combination of thymol, eucalyptol, methyl salicylate and menthol dissolved in ethanol, a surfactant and two co-solvents, particularly propylene glycol and glycerin.

5. Singer is related to oral care compositions containing a selective histamine-2 receptor antagonist compound. The compositions are used for the prevention and treatment of gingivitis or periodontitis. Singer discusses a variety of optional components for use in its oral care compositions, including non-steroidal anti-inflammatory agents.

6. Giorgetti is related to pharmaceutical compositions including nimesulide as an active substance. Nimesulide is a non-steroidal anti-inflammatory drug. The compositions are in the form of liquids, tinctures or mouthwash solutions. The compositions are indicated for the treatment of inflammation of oral and rhinopharyngeal tissue.

7. The subject invention is directed to oral compositions including a combination of an NSAID and four oils, particularly thymol, methyl salicylate, menthol and eucalyptol (hereinafter the "four essential oils"). This combination has anti-inflammatory properties, as disclosed in the specification of the subject application.

8. I conducted experiments in association with the invention of the subject application to demonstrate the anti-inflammatory properties of the combination of the four essential oils contained in the claimed compositions. In particular, the anti-inflammatory properties of the four essential oils were tested by their ability to inhibit an inducible form of prostaglandin E synthase, particularly mPGES-1.

9. The mPGES-1 enzyme is important in the inflammatory pathway. In particular, the inflammatory pathway begins with an inflammatory stimulus, which leads to the production of upregulation of cyclooxygenase-2 (COX-2) and mPGES-1. Arachidonic acid is released upon an inflammatory stimulus by Phospholipase A2 and is converted to the intermediate PGH_2 by COX-2. mPGES-1 then converts PGH_2 to the inflammatory mediator PGE_2. PGE_2 is an essential mediator of inflammation and inflammatory pain. Accordingly, inhibiting mPGES-1 can lead to reduced levels of PGE_2, and thus, less inflammation.

10. The data obtained from this experiment evidences that the combination of the four essential oils, i.e., thymol, methyl salicylate, menthol and eucalyptol, is synergistically effective in inhibiting mPGES-1, and thus, may be effective against inflammation in the oral environment. In particular, the combination of the four essential oils is more than 10 times more effective than the oils individually. Such anti-inflammatory properties exhibited by the four essential oils in this experiment were unexpected.

EXPERIMENTAL

11. An mPGES-1 synthase assay was developed to test the following compounds: methyl salicylate; eucalyptol; menthol; thymol; and the combination of the four essential oils. For comparative purposes, the following commonly known anti-microbial agents and antibiotics also were tested in the assay: Irgasan (triclosan) (anti-microbial); doxycycline hyclate (antibiotic); minocycline HCl (antibiotic); tetracycline HCl (antibiotic); amoxicillin (antibiotic); and ciprofloxacin (antibiotic).

12. For this assay, human mPGES-1 was obtained by expressing the human gene in insect cells. Each of the test compounds was incubated with the recombinant mPGES-1 for 10 minutes. The reaction was initiated with the addition of PGH_2 at a concentration of 2 uM and was allowed to proceed for 60 seconds at room temperature. The reaction was stopped by the addition of $FeCl_2$, which converts any unreacted PGH_2 to PGF_2a.

13. The PGE_2 product was quantified using enzyme linked immunosorbant assay (ELISA) to determine the inhibition of mPGES-1 by each test compound. The inhibitory activity of each compound is represented by its IC_{50} value, which is the concentration of the compound required for 50% inhibition of the enzyme. The results are set forth in Table 1 below.

TABLE 1

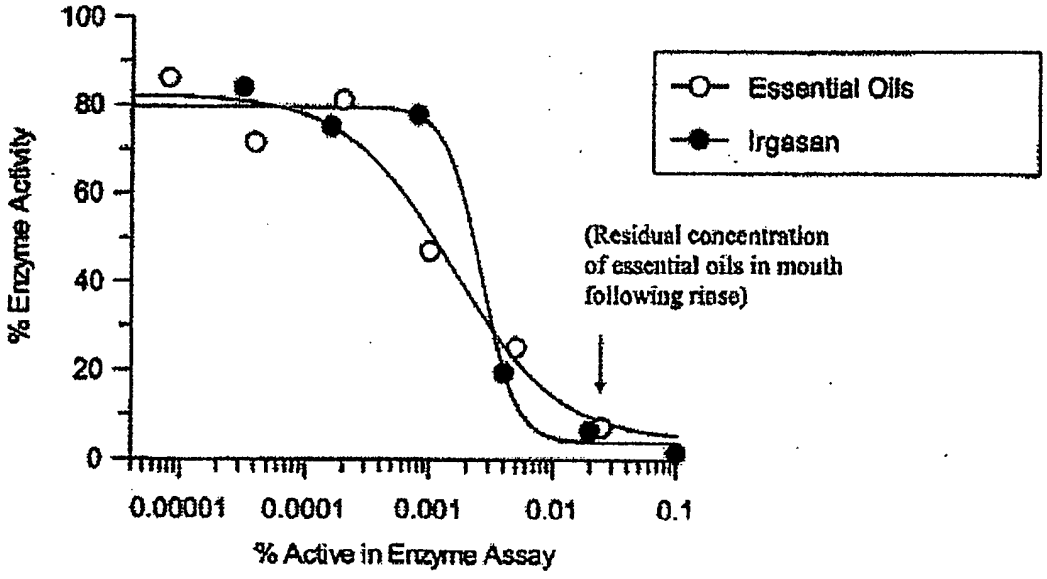
Compound	IC_{50} (% active ingredient) Mean
Methyl salicylate	>1
Eucalyptol	0.5
Menthol	>0.10
Thymol	0.05
Four essential oils	0.00412
Irgasan (triclosan)	0.0025
Doxycycline hyclate Minocycline HCl Tetracycline HCl Amoxicillin Ciprofloxacin	>1

14. As can be seen from the results in Table 1 above, the IC_{50} of mPGES-1 inhibition by each of the four essential oils individually showed hierarchical activity. Thymol showed greater activity than menthol, i.e., a lower level of thymol was required to inhibit 50% of the enzyme, which showed greater activity than eucalyptol, which showed greater activity than methyl salicylate.

15. The combination of the four essential oils, however, exhibited synergistic inhibitory activity with an IC_{50} of mPGES-1 inhibition of 0.00412%. This level was significantly lower (more than 10 times lower) than any of the essential oils individually.

16. The residual level of the four essential oils expected to be found in the mouth following a single 30 second rinse with a mouthwash containing the four essential oils is 0.025%. Therefore, the IC_{50} of the four essential oils demonstrated by this experiment (0.00412%) was significantly below the residual level of the four essential oils expected to be found in the mouth following a typical single rinse. This indicates that a typical, single rinse with a mouthwash containing the four essential oils is enough to impart the anti-inflammatory properties thereof.

17. Further, the inhibitory activity of the combination of the four essential oils was similar to that of Irgasan (triclosan), as provided by the data in Table 1 and depicted in the following chart. However, as seen in Table 1, the commonly prescribed oral antibiotics which were tested in this assay (doxycycline hyclate, minocycline HCl, tetracycline HCl, amoxicillin and ciprofloxacin) were not effective, even at significantly higher levels.



18. In view of the results described above, the inhibitory activity of the combination of the four essential oils against mPGES-1 in this experiment was unexpected. The results of this experiment suggest that the combination of the four essential oils may be useful in reducing PGE_2 levels in the oral environment, and thus, have anti-inflammatory properties.

19. I hereby declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Dated this 16 day of January 2007

Pauline C. Pan

Pauline C. Pan